Protective Effect of Placenta Growth Factor (PIGF) Against Hypoxia-Reoxygenation and Serum-Deprivation Induced Apoptosis in Neonatal Rat Cardiomyocytes

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**ABSTRACT**

Placenta Growth Factor (PIGF) has been shown to play a significant role during pathological events such as ischemia. PIGF knockout mice developed normally, but had significantly impaired angiogenic reactions in ischemia and tumor formation (Falo et al. 2002). The role of PIGF will be investigated during apoptosis induced by serum-deprivation and hypoxia-reoxygenation—physiological conditions relevant to ischemia cardiomyopathy. Likewise, serum-deprivation occurs during ischemic conditions common in cardiomyopathy and triggers apoptosis through the mitochondrial path (Bialik et al. 1999). Hypoxia-reoxygenation mimics ischemia-reperfusion, and causes apoptosis by producing intolerable levels of reactive oxygen species. We intend to study the effect of varying PIGF treatments on caspase-dependent apoptosis in neonatal rat cardiomyocytes during serum-deprivation and hypoxia-reoxygenation. Supported by R15-HL73824-01 (KJT)

**METHODS**

**MAT**

**Rat neonatal cardiomyocyte cell culture:** Rat neonatal cardiomyocytes were isolated and cultured as described (Zheng et al. 2001). Purity of cultures was assessed by indirect immunocytochemistry using monoclonal antibodies against sarcomeric myosin (MF-20, Ultra Iowa Hybridoma Center) and pre-BB-like sarcomeric myosin isoform (Sigma). The negative apoptosis control was 20mM Z-VADfmk (Promega), a pan-caspase inhibitor.

**Culture-Treatments:** Hypoxic conditions were established by placing cell cultures in an anoxic chamber (BIBL, and reoxygenated by removing the culture from the anoxic environment. Serum deprivation was achieved by replacing normal 10% FBS DMEM media with DMEM media lacking FBS. The positive apoptosis control was 1mM Staurosporine (Sigma). The negative apoptosis control was 20mM Z-VADfmk (Promega), a pan-caspase inhibitor.

**RESULTS**

**Fold Caspase-3, 7 Activity**

Cardiomyocytes were isolated from neonatal rat and cultured in DMEM/10% FBS. Two days after culturing, treatments were applied. Cardiomyocytes were deprived of serum and treated with PIGF, then compared to normoxic controls. (A) Cardiomyocytes were placed in hypoxic conditions for 6 hours followed by 4 hours of reoxygenation in normoxic conditions (B). Relative Caspase-3 Activity was quantified with Caspase-GLO assay (Promega). Kruskal-Wallis non-parametric statistical test was used to evaluate the significance of the results. The test revealed statistical significance for the Serum-deprivation treatment group (p=0.001). The test did not reveal statistical significance for the Hypoxia-Reoxygenation treatment group.

**SUMMARY AND CONCLUSION**

Effect of Placenta Growth Factor:
1. S-12 hour pretreatment of PIGF (25ng/ml) significantly decreased apoptosis induced by Serum-Deprivation. Serum-Deprivation caused apoptosis levels to rise to an average of 1.84 times normoxic levels and 8-12 hour pretreatment with PIGF decreased apoptosis to an average of 1.14 times normoxic levels.
2. PIGF (25ng/ml) and 8-12 hour pretreatment of PIGF (25ng/ml) decreased apoptosis induced by Hypoxia-Reoxygenation although the results were not statistically significant. Hypoxia-Reoxygenation caused an increase of apoptosis to an average of 1.62 times normoxic levels and treatment with PIGF at an 8 hour pretreatment reduced the level of apoptosis on average to 1.19 and 1.75 times normoxic levels respectively.

Conclusion:
The results of the study showed that a pretreatment of Placenta Growth Factor protected neonatal cardiomyocytes from apoptosis induced by Serum-Deprivation. The results also demonstrated that PIGF tends to protect neonatal cardiomyocytes from apoptosis induced by Hypoxia-Reoxygenation, but the data failed to reach significance due to variability. It is suggested that a pretreatment with PIGF activates a dominant or delayed pro-apoptotic cell death pathway of the reoxygenation of one or more anti-apoptotic genes to protect cardiomyocytes against apoptosis. Supported by R15-HL73824-01 (KJT)